

Picoeukaryote Identification Lab Activity

Objective

There are two objectives in this activity:

1. to learn why scientists use molecular techniques to study picoeukaryotes, and
2. to investigate real DNA sequences from picoeukaryotes.

Additional Materials

None

Background

This activity is based on two of the techniques discussed in the accompanying article, “Good things come in small packages.”

As a quick review:

- Denaturing Gradient Gel Electrophoresis (DGGE)—A technique used to separate different types of picoeukaryotes. It is based on the sequence of DNA. Sequences that are different will denature (break down) at different points on the gel, thus, each band that forms in a lane has a different DNA sequence and is a different type of picoeukaryote.
- DNA Probe for FISH—A strand of DNA with a specific sequence that will attach to any cell with a complementary sequence in your sample. This will make every picoeukaryote with that sequence glow under a microscope so you can count them.

Questions

1. Imagine that you are an oceanographer and have sampled water from off the coast of Hawaii. You separate out the smallest eukaryotic phytoplankton (picoeukaryotes)

and look at them under the microscope. You take the photos shown in Figure 1. How many different types of picoeukaryote were there in this sample? (Hints: Are you sure there is only one type of picoeukaryote per photo? Are you sure that every photo is a different picoeukaryote? Is it even possible to tell?) Explain.

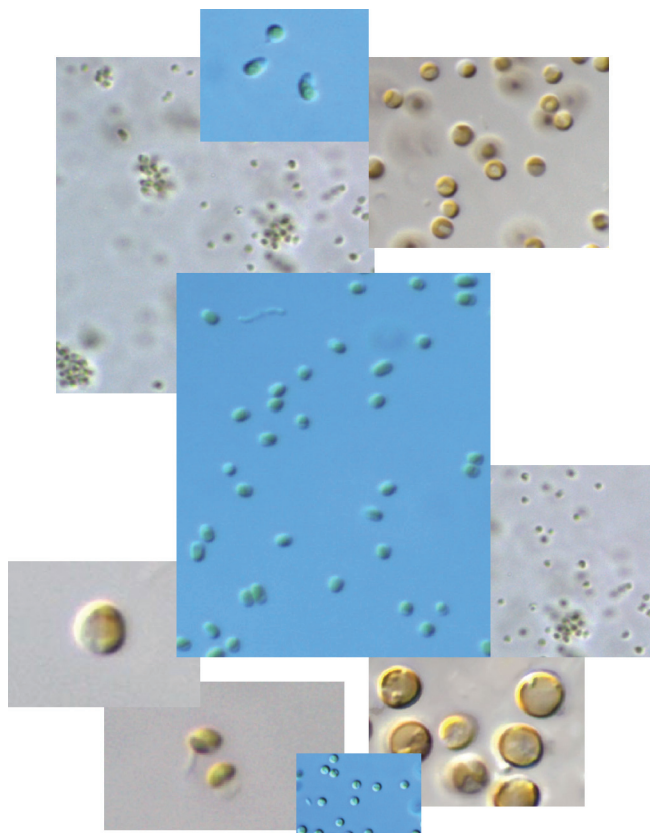


Figure 1: Picoeukaryotes (Pictures courtesy of Provasoli-Guillard National Center for Culture of Marine Phytoplankton—Bigelow Laboratory for Ocean Sciences)

2. To determine how many types of picoeukaryotes are in the water, you decide to run a DGGE (Figure 2). You have sampled from five different depths at this station; the surface of the ocean, 50 m deep, 100 m, 150 m, and 200 m. Based on this gel, what is the total number of different types of picoeukaryotes found at this station? Explain.

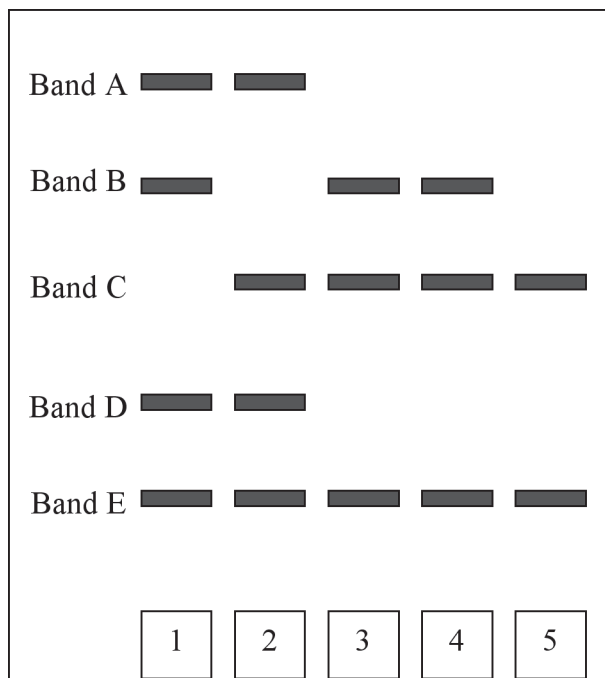


Figure 2: DGGE gel showing the diversity of picoeukaryotes at five different depths. Lane 1: surface, lane 2: 50 m, lane 3: 100 m, lane 4: 150 m, and lane 5: 200 m.

3. According to Figure 2, what can you conclude about the distribution of picoeukaryotes at this station as you move deeper into the ocean?
4. In order to learn more, you decide to sequence the bands so you can compare their DNA (Table 1). You

are particularly interested in knowing if *Ostreococcus* sp. is present at this station—it is the smallest known picoeukaryote and the focus of your research. So, you also acquire the DNA sequence for *Ostreococcus* sp. for comparison. This is available from the National Center for Biotechnology Information, <http://www.ncbi.nlm.nih.gov/>. Just type *Ostreococcus* in the search box once you are on the Web site. Is it possible that *Ostreococcus* sp. is present at your station? Which bands do you think are most closely related to *Ostreococcus* sp.? Which are more distantly related? Explain.

5. What can you propose about the distribution of *Ostreococcus* sp. at this station?
6. If you were to design a DNA probe able to locate all of these types of picoeukaryotes, which section of DNA would be most useful? Explain.
7. If you were to design a probe to identify just those related to *Ostreococcus* sp., which section of DNA would be most useful? Explain

TABLE 1. Comparison of picoeukaryote DNA sequences. DNA sequences from *Ostreococcus* sp. and five bands from the DGGE. The stars (*) indicate regions where all six sequences are the same and the dashes (-) indicate areas where gaps were necessary to align the sequences.

<i>Ostreococcus</i> sp.	TCAGCCTGCTAAATAGTT--GGACCCTACTCTTAGGGCCACA-ACTTCT	bases 1-50
Band A	TCAGCCTGCTAAATAGTT--GTACACTACTCTTAGTGCAGCA-ACTTCT	
Band B	CCCGCCTGCTAAATAGGTGCGGGAATGCGCTTGCAATTGCTGCA-ACTTCT	
Band C	TCAGCCTGCTAAATAGTT--GGACCCTACTCTTAGGGCCACA-ACTTCT	
Band D	CCCGCCTGCTAAATAGCTGTGGGAATGCGCTTGCAATTGCTTCA-ACTTCT	
Band E	CCCGCCTGCTAAATAGTACTGGGAATGC-TTAGCATTGCCAGAGACTTCT	
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<i>Ostreococcus</i> sp.	TAGAGGGACTATGTGCGTGTAGCACGTGGAAGTTTGAGGCAATAACAGGT	bases 51-100
Band A	TAGAGGGACTATGTGCGTTAGCACATGGAAGTTTGAGGCAATAACAGGT	
Band B	TAGAGGGACTTTCGGTGACTAACCGAAGGAAGCTGGGGGCAATAACAGGT	
Band C	TAGAGGGACTATGTGCGTGTAGCACGTGGAAGTTTGAGGCAATAACAGGT	
Band D	TAGAGGGACTTTCGGTGACTAACCGAAGGAAGCTGGGGGCAATAACAGGT	
Band E	TAGAGGGACTTTCGGCGCTAGGCCGAAGGAAGTTGGGGGCAATAACAGGT	
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<i>Ostreococcus</i> sp.	CTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCTACACTGACGAAT	bases 101-150
Band A	CTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCTACACTGACGAAT	
Band B	CTGTGATGCCCTTAGATGTCCTGGGCCGCACGCGCTACACTGGCACAC	
Band C	CTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCTACACTGACGAAT	
Band D	CTGTGATGCCCTTAGATGTCCTGGGCCGCACGCGCTACACTGGCACAC	
Band E	CTGTGATGCCCTTAGATGTCCTGGGCCGCACGCGCTACACTGATGCGT	
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